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GIBBERELLIN A_{28} IN THE FRUITS OF *LUPINUS LUTEUS**

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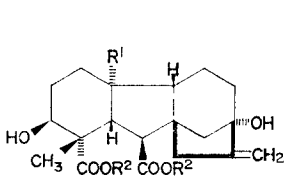
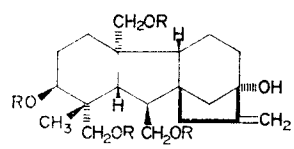
Abstract—A new C_{20} gibberellin, A_{28}, has been isolated from fruits of *Lupinus luteus* and shown to have structure III.

OUR PREVIOUS investigation into gibberellin-like substances in immature fruits of *Lupinus luteus* led to the isolation of two new C_{20} gibberellins, A_{18}[1] and A_{23},[2] for which we have proposed the structures I and II, respectively. An improved method of extraction (see Experimental) and more careful chromatography afforded a third C_{20} gibberellin. We now report the structure of this new gibberellin, which we have named A_{28}.†



	R^1	R^2
I	CH_3	H
II	CHO	H
III	COOH	H
IV	CHO	CH_3
V	$COOCH_3$	CH_3



VI R = H
VII R = $COCH_3$

The i.r. spectrum (KBr) of gibberellin A_{28} indicated the presence of hydroxyls (3430 cm^{-1}) and carboxyls (2800–2400, 1715 sh., 1705 sh. and 1702 cm^{-1}). Methylation of the acid with diazomethane produced a methyl ester, whose i.r. spectrum ($CHCl_3$) showed absorptions attributable to hydroxyls (3590 and 3500–3400 br. cm^{-1}), ester carbonyls (1720 cm^{-1}) and an exocyclic methylene (1662 and 898 cm^{-1}).

In the mass spectrum, the methyl ester exhibited a parent peak at *m/e* 436 in agreement with the formula $C_{23}H_{32}O_8$ and also the prominent peaks at *m/e* 404 (M-32) and 376 (M-60). Simple inspection of its NMR spectra showed the familiar features for gibberellins as summarized in Table 1. Three 3H singlets due to three methoxycarbonyls in these spectra showed the ester to be a trimethyl ester, and the molecular formula, $C_{20}H_{26}O_8$, is assigned to gibberellin A_{28}.

The co-occurrence of gibberellin A_{28} with gibberellin A_{18}(I) and A_{23}(II) as well as the above-mentioned data, suggested that gibberellin A_{28} could have structure III which is

* This study represents a portion of dissertation submitted by H. F. to Kyoto University in partial fulfillment of requirement for the Ph. D. degree, and support in part by Grant to K. K. from Ministry of Education is gratefully acknowledged.

† The trivial name to this new gibberellin has been allocated in agreement with Drs. J. MacMillan and N. Takahashi (cf. *Nature* **217**, 170 (1968).

[1] K. KOSHIMIZU, H. FUKUI, T. KUSAKI, Y. OGAWA and T. MITSUI, *Agri. Biol. Chem.* **32**, 1135 (1968).
[2] K. KOSHIMIZU, H. FUKUI, M. INUI, Y. OGAWA and T. MITSUI, *Tetrahedron Letters* 1143 (1968).

TABLE 1. NMR (δ) OF GIBBERELLIN A_{28} METHYL ESTER

$CDCl_3$	d_5-Pyridine	$\Delta\delta$*	Assignment
1·22 (3H, s.)	1·67	0·45	C–1 CH_3
1·76 (2H, s.)			C–2, 7 OH x 2
2·58 (1H, d., J=12·5 Hz)	3·22	0·64	C–10a H
3·78 (1H, d., J=12·5 Hz)	4·28	0·50	C–10 H
3·62 (3H, s.)	3·63		}
3·68 (3H, s.)	3·68		} $COOCH_3$ x 3
3·73 (3H, s.)	3·73		}
3·99 (1H, m.)	4·40	0·41	C–2 H
4·91 (1H, m.)	5·05	(0·14)	}
5·14 (1H, t., J=2·5 Hz)	5·53	0·39	} C–8 $C=CH_2$

*$\Delta\delta$ values (δd_5-pyridine − $\delta CDCl_3$) in gibberellin derivatives were correlated with their structural features by Hanson.[3]

considered to be biosynthesized from gibberellin A_{18} through A_{23} by oxidation of C-4a substituent on gibbane ring to a carboxyl group.

Gibberellin A_{28} methyl ester(V) was reduced with $LiAlH_4$ in dry dioxane to give a pentaol(VI), whose i.r. spectrum showed no bands characteristic of carbonyls. Acetylation of the pentaol with acetic anhydride-pyridine gave a tetraacetate(VII).

The identity of the pentaol in all respects (TLC, mixed m.p. and i.r. spectrum) with the reduction product of gibberellin A_{23} methyl ester(IV) with $LiAlH_4$ in dry dioxane, permitted us to assign unambiguously structure III for gibberellin A_{28}.

Gibberellin A_{28} and the pentaol(VI) exhibited no activity on the growth of rice seedlings by the method previously reported.[4]

EXPERIMENTAL

M.ps were determined on a hot stage and are uncorrected. I.r. spectra were recorded on a Shimazu AR 275 spectrometer and were calibrated with the 2924, 1601·4 and 1028 cm^{-1} bands of polystyrene. Mass spectra were recorded on a Hitachi RMU-6D mass spectrometer (direct inlet system, 80 eV) and NMR spectra on a Varian A-60 spectrometer. Chemical shifts in the NMR spectra are expressed in ppm from tetramethylsilane as an internal standard and coupling constants in Hz. Singlet, doublet, triplet and multiplet are abbreviated to s., d., t. and m., respectively. Optical rotations were measured with a Yanagimoto photomagnetic direct reading polarimeter Model OR-20. The following chromatographic materials were used: granular charcoal (activated charcoal for chromatography, Wako Pure Chemical, Tokyo), and silicic acid (Mallinkrodt, U.S.A.). Celite 545 was washed successively with distilled water and acetone, and then dried at 100° for 5 hr before use.

Extraction and Isolation of Gibberellin A_{28}

Immature fruits (60 kg) of *Lupinus luteus* were steeped in MeOH (120 l.) for several weeks at room temp. and then filtered. This extraction was repeated with another fresh portion of MeOH. The MeOH extracts were combined and their volume was reduced to 2 l. The aqueous concentrate, after adjustment to pH 3 with 6 N HCl (180 ml), was extracted with benzene (4 × 2 3 l.) to remove soluble materials. To the resulting aqueous layer, charcoal (200 g) was added and the mixture was stirred for 1 hr at 0°. The charcoal was then collected by filtration and washed with water until the washing became neutral. This charcoal treatment was repeated twice. Substances adsorbed on the charcoal were eluted with 70% aqueous acetone (7 × 2·5 l.) and the eluant was removed to give a viscous residue (113 g).

This residue was chromatographed on granular charcoal, and the fractions (8·1 g) eluted with water containing 45–50% acetone were rechromatographed on silicic acid–Celite (1:2). The eluates with 80–100% EtOAc in benzene gave a partially crystalline gum (527 mg), which was further purified by partition

[3] J. R. HANSON, *J. Chem. Soc.* 5036 (1965).
[4] K. KOSHIMIZU, H. FUKUI, T. KUSAKI, T. MITSUI and Y. OGAWA, *Agri. Biol. Chem.* **30**, 941 (1966).

chromatography using *n*-BuOH–benzene mixture increasing *n*-BuOH content on a column of Sephadex LH_{20}–Celite (1:1) impregnated with 1 M phosphate buffer (pH 5·4). Proportions of *n*-BuOH in benzene were controlled by the R_f values of the eluates on TLC of silica gel G (benzene–*n*–BuOH–AcOH, 70:25:5). Fraction (38-40% *n*-BuOH in benzene), giving a purple fluorescent spot (R_f 0·26, R_{GA_3} 0·54) under u.v. light on the plates heated at 120° for 3 min after spraying with 5% H_2SO_4–EtOH, were combined. Recrystallization from acetone–EtOAc-*n*-hexane gave gibberellin A_{28} (115 mg) as colourless rods, m.p. 224–5° (dec.) (Found: C, 60·74; H, 7·17. $C_{20}H_{26}O_8$ requires; C,60·90; H, 6·64%), $[\alpha]_D^{12}$ −6·8° (*c*, 1·18, EtOH), $\delta_{TMS}^{d_5\text{-pyridine}}$ 2·02 (3H, s.), 3·55 (1H, d., J=12·5 Hz), 4·73 (1H, m.), 4·98 (1H, d., J=12·5 Hz), 5·06 (1H, m.) and 5·56 (1H, m.).

Treatment of gibberellin A_{28} with ethereal CH_2N_2 gave a gum, which was purified by chromatography on silicic acid. Elution with benzene–EtOAc gave gibberellin A_{28} methyl ester(V) as an intractable gum, R_f0·46 and $R_{GA_3Me\text{-ester}}$ 1·96 on silica gel G (EtOAc–benzene, 3:2).

$LiAlH_4$ reduction of Gibberellin A_{23} Methyl Ester (IV)

The ester (30 mg) in dry dioxane (5 ml) was refluxed with $LiAlH_4$ (125 mg) for 5 hr. Water was cautiously added and the cooled mixture was filtered to remove the inorganic material. The filtrate was concentrated and chromatographed on silicic acid. Elution with MeOH–CH_2Cl_2 gave a pentaol (VI) (10 mg) which crystallized from MeOH–EtOAc as colourless prisms, m.p. 257–9° (Found: C, 68·14; H, 9·28. $C_{20}H_{32}O_5$ required: C, 68·15; H, 9·15%), ν_{max}^{KBr} 3300 br. and 876 cm^{-1}.

$LiAlH_4$ reduction of Gibberellin A_{28} Methyl Ester (V)

Reduction of the ester(V) by the same method as above gave an alcohol (5 mg), which is identical (TLC, mixed m.p. and i.r. spectrum) with VI.

Acetylation of the Pentaol (VI)

Acetylation of the pentaol(VI) (10 mg) with Ac_2O–pyridine yielded a gum, which was purified by chromatography on Florisil. Elution with benzene–EtOAc gave a tetraacetate(VII) (10 mg) as an intractable gum, $\delta_{TMS}^{CDCl_3}$ 1·03 (3H, s.) 1·71 (1H, s.), 2·10 (12H, s.), 4·05–4·50 (6H, broad signal), 4·95 (2H, m.) and 5·23 (1H, m.).

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MALVACEAE

FREE CYANIDIN IN FLOWERS OF *HIBISCUS MUTABILIS*

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Abstract—The pink basal blotch in petals of *Hibiscus mutabilis* is due to the presence of cyanidin. This may be the first unequivocal case of free anthocyanidin occurring in flowers.

Hibiscus mutabilis L. is a common ornamental shrub of tropical and subtropical regions. It is known by a variety of vernacular names that arise from the conspicuous change in flower colour; from white in the morning to red by late afternoon. Earlier workers have claimed that the red pigment was cyanidin-3,5-diglucoside,[1] but investigation of the flowers in this laboratory failed to reveal any trace of this compound and the major pigment was identified as cyanidin-3-sambubioside.[2]

[1] M. N. SWAMY and S. S. SUBRAMANIAN, *Curr. Sci. India* 33, 112 (1964).
[2] J. B. LOWRY, *J. Sci.* in press.